UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

DEC 0 1 2010

MEMORANDUM

SUBJECT: C5 Honeysweet Plum containing the coat protein gene of plum pox virus.

TO: Denise Greenway, M.S., Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511P)

FROM: Joel V. Gagliardi, Ph.D., Microbial Ecologist
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511P)

THROUGH: John L. Kough, Ph.D., Senior Scientist
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

ACTION REQUESTED: Review data on detection of C5 HoneySweet Plum DNA.

CONCLUSION: Enforcement Analytical PCR Method – **ACCEPTABLE** using the following primers and when performed in triplicate reactions with an internal positive control:

Primer 1 - (plum): 5'-TTATTTCAACGCCAGTCCTGTCCC-3'
Primer 2 - (35S): 5'-GGTAGTTCCCACTGAATCAAAGGC-3'

DATA REVIEW RECORD

Active Ingredient:	Coat protein gene of plum pox virus.
Product Names:	C5 Honeysweet Plum.
Company Name:	U.S. Department of Agriculture, Agricultural Research Service.
EPA Reg. Nos.:	11312-8.
Chemical Number:	006354.
Decision Numbers:	442177.
DP Barcodes:	384129.
MRID Nos.:	482841-01.

BACKGROUND:

The submitted draft method used positive (C5 HoneySweet leaf), negative (*Prunus domestica* leaf), no DNA (water) and a ubiquitous plant internal control (cytochrome oxidase / COX) to assess all steps of the protocol, including a dilution series of C5 and non-C5 plum leaf extracts. Expected results include positive results for COX from all plant samples, positive results for C5 from C5 leaf but negative from non-C5 *Prunus domestica* leaf, and negative results for both C5 and COX from the no DNA controls. Dilution assays will report results as a consensus of two replicates, or as "limit of detection" positive or negative for 2/3 or 1/3 asymmetrical results, from 1:100 to 1:10,000 mixtures of C5 and non-C5 leaf tissues, extracted with commercial Qiagen DNeasy Plant MiniKits.

The proposed unique primer set spans the C5 resistance gene and GUS 35s promoter.

C5 Forward 5'-GTGCATTGCAGAAGCAAC-3';
35S Reverse-5'CGCAATGATGGCATTTGTAGG-3'

Study Type: Enforcement Analytical Method (OCSPP 830.1800).
MRID No.: 482841-01.
Test Material: C5 HoneySweet Plum – Resistant to Plum Pox Virus (Plum Pox Viral Coat Protein Gene).
Study Summary: Internal positive controls (Cox gene) were detected in all samples. Negative control samples all showed no reaction to C5 specific PCR, indicating cross contamination of samples did not occur. C5 HoneySweet DNA could be detected when extracted leaf material was at 0.01% in a majority of samples though repeatable detection was made a 0.05% and above when mixing leaf materials with the parent cultivar BlueByrd. This translates to an ability to detect the transgenic variety repeatably (using three replicate reactions) when in a mixed sample of 1 leaf in 2,000 though even at a rate of 1 leaf in 10,000 the detection rate was over 50%. This is sufficient to determine presence of the C5 trait for use in commerce using the following primers:

Primer 1 - (plum): 5'-TTATTTCAACGCCAGTCCTGTCCC-3'
Primer 2 - (35S): 5'-GGTAGTTCCCACTGAATCAAAGGC-3'

Classification: ACCEPTABLE.

DATA EVALUATION RECORD

Review by: Joel V. Gagliardi, Ph.D.
Secondary Review by: John L. Kough, Ph.D.

Study Type	Enforcement Analytical Method (OCSPP 830.1800).
MRID No.	482841-01.
Test Material	C5 HoneySweet Plum – Resistant to Plum Pox Virus (Plum Pox Viral Coat Protein Gene).
Study No.	IR-4 PR # 0377B.
Sponsor	Ralph Scorza, Ph.D.; USDA-ARS, Appalachian Fruit Research Station; Kearneysville, West Virginia.
Testing Facility	USDA-ARS, Appalachian Fruit Research Station; Kearneysville, West Virginia.
Title of Report	The Detection of C5 'HoneySweet' Plum DNA.
Authors	Deepak K. Srivastava, Ph.D.; Ralph Scorza, Ph.D.; Michael Braverman, Ph.D.
Study Completed	October 22, 2010
Study Summary	Internal positive controls (Cox gene) were detected in all samples. Negative control samples all showed no reaction to C5 specific PCR, indicating cross contamination of samples did not occur. C5 HoneySweet DNA could be detected when extracted leaf material was at 0.01% in a majority of samples though repeatable detection was made a 0.05% and above when mixing leaf materials with the parent cultivar BlueByrd. This translates to an ability to detect the transgenic variety repeatably (using three replicate reactions) when in a mixed sample of 1 leaf in 2,000 though even at a rate of 1 leaf in 10,000 the detection rate was over 50%. This is sufficient to determine presence of the C5 trait for use in commerce using the following primers: Primer 1 - (plum): 5'-TTATTTCAACGCCAGTCCTGTCCC-3' Primer 2 - (35S): 5'-GGTAGTTCCCACTGAATCAAAGGC-3'
Classification	**ACCEPTABLE.**
Good Laboratory Practice	Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. PCR primers and Taqman probe:

Primer 1 - (plum): 5'-TTATTTCAACGCCAGTCCTGTCCC-3'
Primer 2 - (35S): 5'-GGTAGTTCCCACTGAATCAAAGGC-3'
Taqman double quenched fluorescent probe:
5'-6-carboxyfluorescein/TGGCAAGGA/ZEN quencher/AATGTGCGAGTTCTGT/Iowa Black quencher-3'

B. DNA Extraction: Three independent batches were extracted three times each.

C. Scoring: If two of three reps are positive the sample is scored positive.

II. RESULTS

DNA Source Tree (%)		PCR Replicate Results / Cox Control (%)				
HoneySweet	BlueByrd	1	2	3	4	5
100	0	+ / +	+ / +	+ / +	+ / +	+ / +
10	0.01	100 / 100	100 / 100	100 / 100	ND	ND
1	0.05	100 / 100	100 / 100	100 / 100	ND	ND
0.1	0.1	100 / 100	100 / 100	100 / 100	ND	ND
0.05	1	67 / 100	100 / 100	67 / 100	ND	ND
0.01	10	100 / 100	67 / 100	0 / 100	ND	ND
0	100	- / +	- / +	- / +	- / +	- / +
0	0	- / -	- / -	- / -	- / -	- / -

III. CONCLUSION

Internal positive controls (Cox gene) were detected in all samples. Negative control samples all showed no reaction to C5 specific PCR, indicating cross contamination of samples did not occur.

C5 HoneySweet DNA could be detected when extracted leaf material was at 0.01% in a majority of samples though repeatable detection was made a 0.05% and above when mixing leaf materials with the parent cultivar BlueByrd. This translates to an ability to detect the transgenic variety repeatably (using three replicate reactions) when in a mixed sample of 1 leaf in 2,000 though even at a rate of 1 leaf in 10,000 the detection rate was over 50%. This is sufficient to determine presence of the C5 trait for use in commerce.

DEFICIENCIES: None.